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Antimicrobial and Antioxidant Activity of Trikatu Syrup Formulated by Classical and Modern Method

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ABSTRACT

Trikatu, a Sanskrit word meaning three acrids' is an Ayurvedic formulation consisting of powders of three drugs black pepper *(Piper nigrum, Linn.)* long pepper *(Piper longum, Linn.)* and ginger *(Zingiber officinalis, Rosc.)* in equal proportion. Trikatu has gained importance in the traditional system of medicine due to its chief alkaloidal constituent. Trikatu syrup is widely used for the treatment of fever, asthma, cold and cough, diabetes, nasal diseases, obesity, anorexia, digestive, respiratory system and normal urinary tract function. The trikatu syrup improves digestion, supports normal gastric function, and normal circulation, maintains a healthy immune system. To increase bioavailability of drug and nutrients, reduction in HCl secretion and increase in gastrointestinal tract (GIT) blood supply. Pharmacological activities of trikatu syrup reported include antioxidant, analgesic, anti-inflammatory and antimicrobial activity. While keeping the Ayurvedic principles identical to traditional approaches, we can investigate new technologies or analyse technologies that already exist in order to enhance the dosage forms and scaling up of the medicine in terms of quality and quantity. Therefore, the study is planned to formulation and evaluation of trikatu syrup. The results obtained by this study can really provide crucial information for physicians to determine the accurate actions and dosage of this drug in various clinical manifestations.

INTRODUCTION OF OBESITY METABOLISM SYNDROME

Obesity is a complex disease involving an excessive amount of body fat. Obesity isn't just a cosmetic concern. It's a medical problem that increases the risk of other diseases and health problems, such as heart disease, diabetes, high blood pressure and certain cancers. Metabolic syndrome is a cluster of conditions that occur together, increasing your risk of heart disease, stroke and type 2 diabetes.

Co-relation of obesity, metabolism, inflammation and diabetics

Obesity is associated with low-grade inflammation of white adipose tissue (WAT) resulting from chronic activation of the innate immune system and which can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes.

In obesity, WAT is characterized by increased production and secretion of a wide range of inflammatory molecules including TNF-αand interleukin-6 (IL-6), which may have local effects on WAT physiology but also systemic effects on other organs. Recent data indicate that obese WAT is infiltrated by macrophages, which may be a major source of locally-produced pro-inflammatory cytokines. Interestingly, weight loss is associated with a reduction in the macrophage infiltration of WAT and an improvement of the inflammatory profile of gene expression. Several factors derived not only from adipocytes but also from infiltrated macrophages probably contribute to the pathogenesis of insulin resistance.

Most of them are overproduced during obesity, including leptin, TNF-α, IL-6 and resistin. Conversely, expression and plasma levels of adiponectin, an insulin-sensitising effector, are down-regulated during obesity. Leptin could modulate TNF-α production and macrophage activation. TNF-α is overproduced in adipose tissue of several rodent models of obesity and has an important role in the pathogenesis of insulin resistance in these species. However, its actual involvement in glucose metabolism disorders in humans remains controversial. IL-6 production by human adipose tissue increases during obesity.

It may induce hepatic CRP synthesis and may promote the onset of cardiovascular complications. Both TNF- α and IL-6 can alter insulin sensitivity by triggering different key

steps in the insulin signaling pathway. In rodents, resistin can induce insulin resistance, while its implication in the control of insulin sensitivity is still a matter of debate in humans.

Adiponectin is highly expressed in WAT, and circulating adiponectin levels are decreased in subjects with obesity-related insulin resistance, type 2 diabetes and coronary heart disease. Adiponectin inhibits liver neo glucogenesis and promotes fatty acid oxidation in skeletal muscle. In addition, adiponectin counteracts the pro-inflammatory effects of TNF-αon the arterial wall and probably protects against the development of arteriosclerosis. In obesity, the pro-inflammatory effects of cytokines through intracellular signaling pathways involve the NF-kB and JNK systems. Genetic or pharmacological manipulations of these effectors of the inflammatory response have been shown to modulate insulin sensitivity in different animal models. In humans, it has been suggested that the improved glucose tolerance observed in the presence of thiazolidinediones or statins is likely related to their anti-inflammatory properties. Thus, it can be considered that obesity corresponds to a sub-clinical inflammatory condition that promotes the production of pro-inflammatory factors involved in the pathogenesis of insulin resistance.

Drug Profile

A. LONG PEPPER

Fig. 1: Dried *Piper longum Linn*

Scientific Name: *Piper longum L.*

Biological source: It consists of dried flowering vine of *Piper longum Linn.*

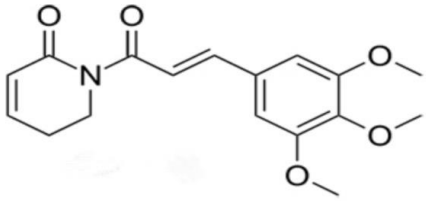
Family: *Piperaceae*

Synonyms: Piper latifolium Hunter.

Chemical constituents: Piperine is the major and active constituent of long pepper (*Piper longum).* The piperine content is 3-5% (on dry weight basis) in *P. longum*. The fruits of p.longum containg large no of Alkaloids, Amides, Ligands, Ester, Volatile oil. Volatile oil of the fruit *P. longum* is a complex mixture. Major components of essential oil are caryophyllene and pentadecane (both about 17.8%) and bisaboline (11%) along with volatile piperine. Other components include thujine, terpinoline, p-cymene, p-methoxy acetophenone, and dihydrocarveol.

Chemical properties

- **Chemical names:** (E)-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one
- **Molecular weight:** 285.33
- **Molecular formula:** $C_{17}H_{19}NO_3$



Uses: Anticancer, Antiplatelet, anti-inflammatory, antioxidant, immunomodulatory, analgesic.

B. BLACK PEPPER

Fig. 2: Dried unripe fruits of *Pipper nigrum Linn*

Scientific Name: *Piper nigrum*

Biological source: It consists of dried unripe fruits of *Pipper nigrum Linn.*

Family*:* *Piperaceae.*

Synonyms: Kalimirch (Hindi); Golmarich (Bangali); Milagu- Milagu.

Geographical Source: South India, Indonesia, Brazil, West Indies, Malaysia and Sri Lank

Chemical Constituents: *P.nigrum* contains lignans, alkaloids, flavonoids, amides, and other aromatic compounds along with approximately 3.5% of volatile oil. Components of essential oil include sabinene, pinene, linalool, limonene, and phellandrene. Piperine is an alkaloid and the chemical marker of P. nigrum. Chavicine which is an isomer of piperine is also present. Piperine and Chavicine are not responsible for the aroma of the black pepper. Piperine is responsible for pungency of the black pepper.

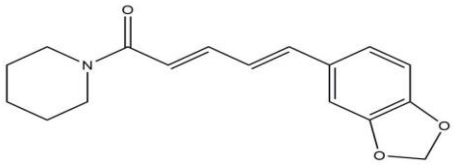
Chemical properties :

- **Chemical names:** a. 1- piperoyl piperidine

  b. (E,E)1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine

- **Molecular weight:** 285.33

- **Molecular formula:** $C_{17}H_{19}NO_3$



Uses

Carminative, Stimulant, Stomachic, Condiment & Spice , Stimulate the flow of gastric juice.

C. DRY GINGER

Fig. 3: dried rhizomes of the *Zingiber officinale Roscoe.*

Scientific Name: *Z. officinalis*

Biological Source: Ginger consists of the dried rhizomes of the *Zingiber officinale Roscoe.*

Family: *Zingiberaceae.*

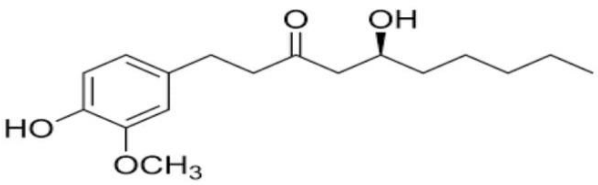
Synonym: Rhizoma zingiberis, Zingibere

Geographical Source: It is mainly cultivated in West Indies, Nigeria, Jamaica, India, Japan, and Africa.

Chemical Constituents: Ginger contains 1 to 2% volatile oil, 5 to 8% pungent resinous mass and starch. The volatile oil is responsible for the aromatic odour and the pungency of the drug is due to the yellowish oily body called gingerol which is odourless. Volatile oil is composed of sesquiterpene hydrocarbon like α-zingiberol; α-sesquiterpene alcohol α-bisabolene, α-farnesene, α-sesquiphellandrene. Less pungent components like gingerone and shogaol are also present. Shogal is formed by the dehydration of gingerol and is not present in fresh rhizome.

Chemical properties:

- **Molecular formula**:$C_{35}H_{52}O_6$
- **Molecular formula**:568.8
- **Chemical Name**: (E)-1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one;1-(4-hydroxy-3-methoxyphenyl)-5-methyldecan-3-one



Uses: Ginger is stomachic stimulant and aromatic carminative.

MATERIALS AND METHOD

Materials

Drug:

The drugs such as Long pepper, Black pepper, Dry ginger, Trikatu powder were procured from local market.

Chemicals:

The list of chemicals is given in table no 1.

Table no. 1: List of Chemicals

Sr. no.	Chemicals
1.	Molisch reagent
2.	Fehling solution A
3.	Fehling solution B
4.	Mayer's reagent
5.	Dragendorff's reagent
6.	Wagner's reagent
7.	Barfoed's reagent
8.	Benedict's reagent
9.	Sulphuric acid
10.	Chloroform
11.	Hydrogen peroxide
12.	Ascorbic acid
13.	Phosphate buffer

Apparatus and instruments:

The apparatus and instruments used as Soxhlet apparatus, heating mantle, RBF, UV-Vis spectrophotometer (Shimadzu, W 1900i).

METHODS

Pharmacognostical Evaluation of Raw Material

- **Macro-morphology study**:

Macroscopic or organoleptic characterization such as color, odor, taste, and texture of powder was performed using standard methods.

- **Microscopic powder study:**

The pepper powder microscopy was performed according to the standard method. A pinch of powder was mounted with few drops of iodine solution (5%) on a microscopic slide and then observed under a microscope. The same procedure a pinch of ginger powder was mounted with few drops of Phloroglucinol + Conc. HCl and Iodine solution on a microscopic slide and then observed under a microscope.

- **Microscopic characteristics:**

Quantitative microscopy of the transverse sections (T.S) and fruit powder were performed to determine the size and dimensions of tissues, cells and cell contents.

Phytochemical Test

1) Test for Alkaloids

Mayer's test:

Add 1 ml drug powder add 2 ml Mayer's reagent yellowish white precipitate observed then alkaloids are present.

Dragendorff 's test:

Add 1 ml drug powder add 1 ml Dragendorff's reagent orange brown precipitate observed then alkaloid are present.

Wagner's test:

Add 1 ml drug powder add 2 ml Wagner's reagent red precipitate observed then alkaloid are present.

2) Test for carbohydrates

Molisch test:

Add 2 ml aqueous sample and add 5 drops of Molisch reagent in test tube, add gently through the side by tilting the test tube 2 ml of conc. H_2SO_4 so at to form bottom layer two liquid layer are observed then carbohydrates are present.

Fehling test:

Add 2 ml of Fehling solution A and 2 ml of Fehling solution B and add 2 ml of liquid solution then boil it. Red precipitate observed then carbohydrates are present.

Barfoed's test:

Add 2 ml of Barfoed's reagent and 2 ml of liquid solution. Boil on water bath for 2 min then cool it. Brick red precipitate are observed then carbohydrates are present.

Benedict's test:

Take 5ml of benedict's reagent and add 8 drops of liquid solution then boil for 2min allow to cool green precipitate observed then carbohydrate are present.

3) Flavonoids test:

Add drug powder and sulphuric acid deep yellow precipitate observed then flavonoids are present.

Add extract of drug and add sodium hydroxide yellow color observed then flavonoid are present.

4) Steroids test: Add 1mg extract of drug and add 10ml chloroform and conc. Sulphuric acid to test tube by side yellow fluorescent form then steroid are present.

Method of Preparation:

Formulation-1

- **Method of preparation of decoction:**

Weight accurately 16.6 gm of each herbal ingredient. Then cut into small pieces and added into 100ml of water. The mixture was boiled until total volume become one fourth of initial volume. Decoction was cooled and filtered. Filter was taken to prepare final herbal syrup.

- **Method of preparation of simple syrup:**

66.7 g of Sucrose was weighed and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 100 ml.

- **Method of preparation of final herbal syrup**:

one part of decoction was mixed with five parts of simple syrup (1:1). Required quantity of Sodium benzoate (0.2%) was added as preservative to the above mixture. The final herbal syrup was then subjected for evaluation and final activity.

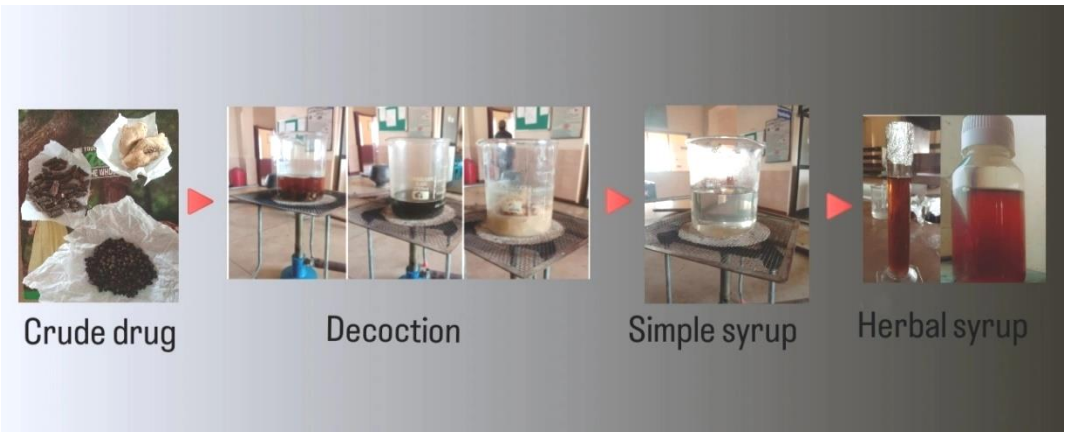


Fig.4: Preparation of herbal syrup by using decoction

Table no. 2.1: Formula for preparation of herbal syrup by using decoction method (50 ml)

Sr. No.	Ingredient	Quantity	Role
1.	Long pepper	5.5 ml	API
2.	Black pepper	5.5 ml	API
3.	Ginger	5.5 ml	API
4.	Sugar	33.3 ml	Sweetener
5.	Sodium benzoate	0.2 gm	Preservative

Formulation-2

- **Extraction of crude drug**

The 20gm of crude drug powder was extracted with 90% ethanol at 50-60°c in a Soxhlet apparatus. The different extracts were collected in separate container and concentration to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°c) and note down the yield of crude extracts.

- **Extraction of trikatu power:**

The 100gm of trikatu extracted with 90% ethanol at 50-60°c in a soxhlet apparatus. The extracts were concentration to dryness in water bath controlled temperature (50-60°c). The dried 90% of ethanolic extract dissolved on known volume of distilled water.

- **Preparation of syrup extraction:**

The syrup was prepared by dissolving API in propylene glycol at 40-50°c then state amount of glycerin and sorbitol were added in it. Dissolve preservatives in amount of boiled and cooled water. The given amount of thickening agent was added and mixed thoroughly. The volume was made up to 50 ml with boiled and cooled water.

Fig.5: Preparation of extract

Table no. 2.2: Formula for preparation of herbal syrup by using Extraction method (50ml)

Sr. No.	Ingredient	Quantity	Role
1.	Trikatu extract	12.5 ml	API
2.	Propylene glycol	20 ml	Solublizer
3.	Sodium benzoate	0.2%	Preservative
4.	Sorbitol	5ml	Sweetener agent and stabilizer
5.	Glycerin	7.5ml	Thickener/ Diluent
6.	Distilled water	Q.S to 50%	Vehicle

Pharmacological study

Antimicrobial Activity:

1) The antimicrobial activity ethanol extract of trikatu was evaluated by agar well diffusion method. Trikatu extract showed strong antibacterial activity against *E.coli*. Streptomycin sulphate was used as positive control (10mcg/disc).The antibacterial activities of ethanol extracts of trikatu were performed against enteric bacterial pathogens such as *E.coli*. Ethanol extract of trikatu showed moderate antibacterial activity against all bacterial pathogens.

2) In another experiment antibacterial activity of ethanol extract of trikatu herbal formulation had been performed against *E.coli* using agar well diffusion method. Ethanolic extract of trikatu showed moderate activity against tested microbes as compared to antibiotic syrup used positive control.

Antioxidant activity:

Hydrogen Peroxide Scavenging (H_2O_2) Assay

1. A solution of hydrogen peroxide (40mM) is prepared in phosphate buffer (50mM pH 7.4).

2. The concentration of hydrogen peroxide is determined by absorption at 230nm using a spectrophotometer.

3. Extract (20-60 micron/ml) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.

4. Take ascorbic acid as a standard and follow the procedure above. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenged H2O2} = \frac{(\text{Absorbance control} - \text{Absorbance test})}{\text{absorbance control}} \times 100$$

RESULT AND DISCUSSION

- **Macro-morphology study**:

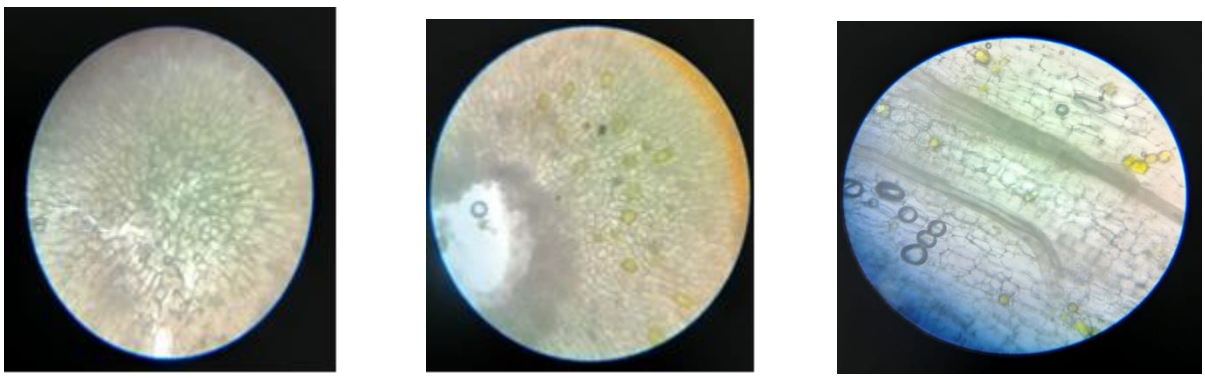
All the raw material were procured from local market, evaluated for their organoleptic characteristics including colour, odour, taste, size, shape is given in table no. 3.

Table no. 3: Organoleptic Evaluation of Raw Material

Organoleptic characteristics	**Long pepper**	**Black pepper**	**Dry ginger**
Colour	Grayish brown	Blackish grey	Crowned with brownish soft fibers.
Odour	Characteristics	aromatic	Pleasant or characteristics
Taste	Pungent	pungent	Pungent
Size	0.2-0.4 cm in diameter.2-3 cm in length	0.4-0.5cm in diameter	2.5-7.5cm in length
Shape	Cylindrical	Globular or oblong	Irregular or nodule

- **Microscopical characteristics:**

The microscopic evaluation of was carried out for fruit of *Piper longum Linn, Piper nigrum Linn* and root part of *Zingiber officinale.* Quantitative microscopy of the transverse sections (T.S) were performed to determine the tissues, cells and cell contents. The study involves the determination of cork, cortex, oil cells, fibers, epicarp, mesocarp of the species.



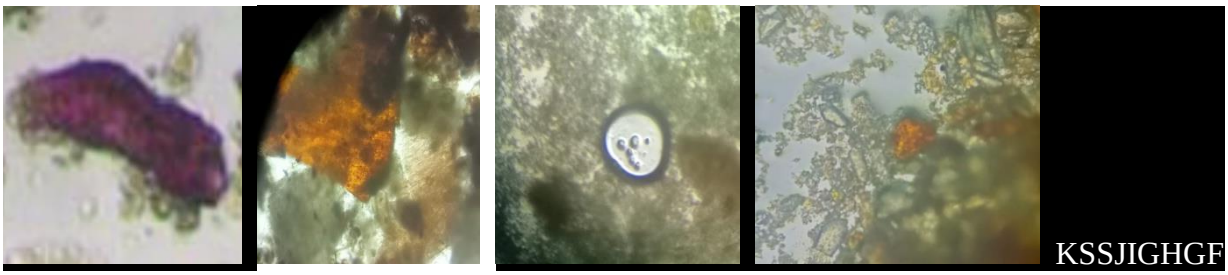
T.S. for long pepper T.S. for black pepper T.S. for dry ginger

Fig.6: T. S. of Raw Materials

➢ **Microscopic powder study:**

A) LONG PEPPER:

The microscopic examination of long pepper powder shows stone cells, brown content, oil globules and oleoresin.

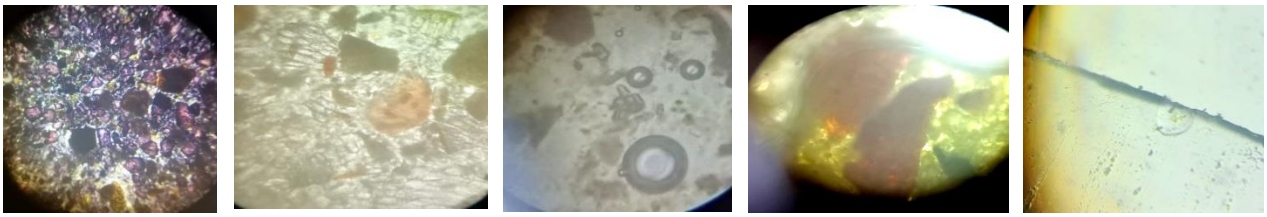


Stone Cell Brown Content Oil Globules Oleoresin

Fig. 7: Microscopic Evaluation of Long Pepper

B) BLACK PEPPER:

The microscopic examination of black pepper shows starch granules, brown content, lignified endocarp, stone cells and oil cells.



Starch granules **Brown Content** **Lignified Endocarp** **Stone Cell** **Oil Cells**

Fig. 8: Microscopic Evaluation of Black Pepper

C) DRY GINGER:

The microscopic examination of dry ginger shows fibers, parenchyma cells and vessels.

Fibers **Parenchyma Cells** **Vessels**

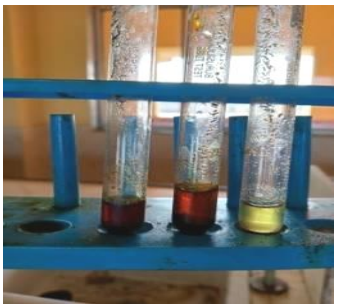
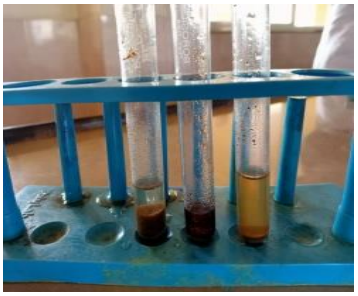
Fig. 9: Microscopic Evaluation of Dry ginger

- **Phytochemical Test**

The preliminary phytochemical observation of crude drug of four different test samples such as Trikatu powder has shown the occurrence of alkaloids, carbohydrates, flavonoids, steroids. It indicates that, the Trikatu powder is a mixture of all three phytoconstituents and interaction of all these chemicals resulted in synergistically enhanced therapeutic efficacy of antibacterial activity.

Table no. 4: Phytochemical Evaluation of Raw Material

Test		**Long pepper**	**Black pepper**	**Dry ginger**	**Trikatu**
Alkaloids	Mayer's test	+	+	+	+
	Dragendorff's test	+	+	+	+
	Wagner's test	+	+	+	+
Carbohydrates	Molisch test	+	+	+	+
	Fehling test	+	-	-	-
	Barfoed's test	+	+	+	-
	Benedict's test	+	+	+	-
Flavonoids	Extract + H_2SO_4	+	-	+	+
	Extract +NaOH	+	+	+	+
Steroids	Extract+ chloroform + conc. H_2SO_4	+	-	+	+


Test for long pepper



Test for black pepper



Test for dry ginger

Methods For preparation of Formulations:

Formulations 1 and 2 were prepared by taking raw material Long pepper, black pepper and dried ginger in the ratio of 1:1:1 using classical and modern method.

Screening of Formulations for their Pharmacological Activities

- **Antimicrobial activity:**

The antibacterial activity was evaluated and diameter of zone of inhibition was measured**.** Ethanol extract of trikatu showed moderate antibacterial activity against all bacterial pathogens.

The trikatu syrup (F1) is more positive effect against E.coli by compared to standard antibiotic syrup (Asthakind ® - DX).

Table no. 5: Data on Antimicrobial Evaluation of Raw materials and Formulations

Drug	**Zone of inhibition in *E.coli***
Long pepper	1.2 cm
Black pepper	1.2 cm
Ginger	1 cm
Trikatu	1.3 cm
Streptomycin sulphate	2 cm
Syrup 1	1.5 cm
Syrup 2	1 cm

Crude drug ethanol extract against *E.coli* **Formulation against *E.coli***

Fig. 10: Zone of Inhibition

Antioxidant activity:

The antioxidant activity was evaluated to determine percentage scavenging activity of drug extract and decoction is significant compared to that of standard ascorbic acid. (Table no. 6) shows more H_2O_2 scavenging activity of extract in comparison with standard ascorbic acid.

- **Antioxidant activity of extract by H_2O_2 assay:**

The study was carried out to evaluate the antioxidant potential of formulations prepared by classical method and modern method. The overall action was found to be good. However the

formulation prepared by modern method was possesses better antioxidant potential than formulation prepared by classical method.

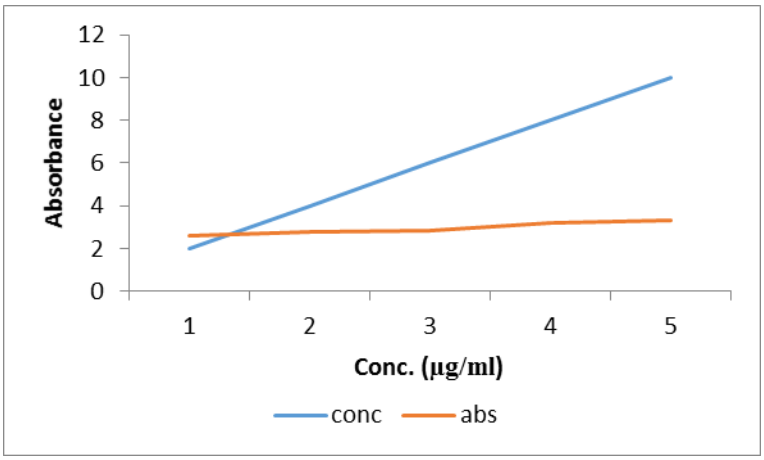


Fig. 11: Antioxidant activity of formulation prepared by Modern method

Table no. 6: Date on Antioxidant activity of formulation prepared by Modern method

Sr. no.	Concentration (µg/ml)	absorbance
1	0.00	0.00
2	2	2.670
3	4	2.802
4	6	2.841
5	8	3.215
6	10	3.334

Antioxidant activity of decoction by H_2O_2 assay

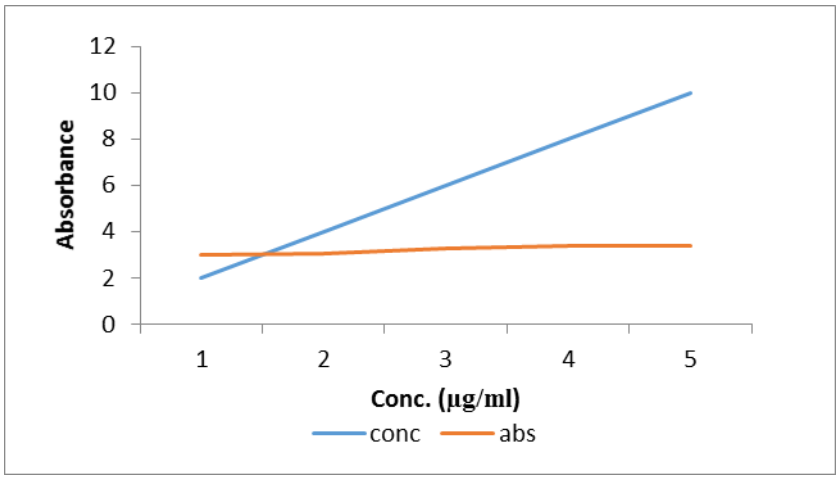


Fig. 12: Antioxidant activity of formulation by Classical Method

Table no. 7: Data on Antioxidant activity of formulation by Classical Method

Sr.no.	Concentration (µg/ml)	absorbance
1	0.00	0.00
2	2	2.994
3	4	3.028
4	6	3.384
5	8	3.369
6	10	3.028

Absorbance for absorbance of extract

Ai- absorbance of control= 0.117

At- absorbance of test= 0.101

%scavenged (H_2O_2) = (At-Ai)/Ai X100

=13.67%

Absorbance for absorbance of decoction

Ai- absorbance of control= 0.188

At- absorbance of test= 0.186

%scavenged (H_2O_2) = (At-Ai)/Ai X100

= 1.063%

CONCLUSION-

By using long pepper, black pepper, dry ginger we successfully prepared the trikatu syrup we have done all the evaluation tests and justified the antimicrobial & antioxidant activity, it has been concluded that the prepared herbal trikatu syrup to determine the accurate actions and dosage of this drug in various clinical manifestations.

For extract absorbance was found to be 0.101& for ascorbic acid which is used as standard was found to be 0.186. Thus from the study it was found that extract has less antioxidant activity than standard L-ascorbic acid. For extract two solutions were prepared of Test and Control and % Scavenging activity was found to be 13.67 and 1.063% respectively. Thus from this study, it may be concluded that formulation is having potent antioxidant action.

Ethanolic extract of crude drug trikatu showed moderate activity against *E.coli*.as compared to antibiotic streptomycin sulphate used as positive control was found to be 1.2 cm (Long pepper), 1.2 cm (Black pepper), 1 cm (Ginger), 1.5 cm (Trikatu extract) respectively and another formulation of trikatu syrup concluded that trikatu syrup (F1) is more positive effect against E.coli by compared to standard antibiotic syrup.

Trikatu formulation was found to possess higher rate of phytoconstituents and promising antibacterial activity. It is also confirmed that, these spicy products triggers natural immune system to fight against enteric bacterial infection.

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